

What is claimed is:

1.

An oligonucleotide sequence which encodes a synthetic suppressor tRNA comprising:

- A) a human tRNA structural gene sequence comprising no more than twenty 3' flanking residues and no 5' flanking residues, said sequence encoding an anticodon region for pairing with mRNA;
- B) a total length of less than 150 nucleotides;
- C) an anticodon sequence which has been modified to recognize a codon different from that which is originally recognized.

2.

A synthetic suppressor tRNA molecule encoded by the oligonucleotide of claim 1.

3.

The oligonucleotide of claim 1 wherein said anticodon region encodes an nonsense mutation selected from the group consisting of:

amber (TAG), ochre (UAA) and opal (UGA).

4.

The oligonucleotide of claim 1 further comprising a second oligonucleotide sequence as described in claim 1 wherein said two sequences are in tandem.

5.

The oligonucleotide of claim 1 wherein said tRNA structural gene sequence encodes a serine tRNA.

6.

The oligonucleotide sequence of claim 1 wherein said tRNA structural gene sequence encodes an arginine tRNA.

7.

The oligonucleotide of claim 1 wherein said oligonucleotide has a sequence selected from the group consisting of SEQ ID NOS:1-10, their complements and their functional equivalents.

8.

A method of restoring translation to a nucleotide sequence which includes a nonsense mutation in a cell comprising:

introducing to said cell a nucleic acid sequence which

encodes a synthetic suppressor tRNA oligonucleotide, said oligonucleotide comprising:

- A) a human tRNA structural gene sequence comprising no more than twenty 3' flanking residues and no 5' flanking residues, said sequence encoding an anticodon region for pairing with mRNA;
- B) a total length of less than 150 nucleotides;
- C) an anticodon sequence which has been modified to recognize a codon different from that which is originally recognized, wherein said anticodon is one which will pair with said nonsense mutation and said tRNA structural gene sequence encodes an amino acid which is deleted by said nonsense mutation.

9.

The method of claim 8 wherein said nucleotide sequence with said nonsense mutation is one which has been introduced to said cell.

10.

The oligonucleotide of claim 8 wherein said tRNA structural gene sequence encodes a serine tRNA.

11.

The oligonucleotide sequence of claim 8 wherein said tRNA structural gene sequence encodes an arginine tRNA.

12.

The oligonucleotide of claim 8 wherein said oligonucleotide has a sequence selected from the group consisting of SEQ ID NOS:1-10, their complements and their functional equivalents.

13.

A method of restoring translation to a nucleotide sequence which includes a nonsense mutation in a cell comprising:  
introducing to said cell a synthetic suppressor tRNA oligonucleotide, said oligonucleotide being one which is encoded by the sequence of claim 1.

14.

A nucleotide vector comprising the nucleotide sequence of claim 1.

15.

The nucleotide vector of claim 14 wherein said vector is a viral vector.

16.

The vector of claim 14 wherein said vector is a viral vector selected from the group consisting of:  
a retroviral, adenoviral, adeno-associated, Herpes simplex virus and Herpes simplex viral vector.

17.

The method of claim 14 wherein said vector is a Herpes virus vector.

18.

The method of claim 14 wherein said vector is a Herpes virus mini amplicon vector comprising:  
an Epstein-Barr virus ori P and EBNA-1 sequence to maintain the plasmid episomally, a hygromycin resistance gene, an HSV-1 lytic replication origin (ori S), and a HSV-1 terminal packaging signal.

19.

The vector of claim 14 wherein said vector is the pHhargsup tRNA<sup>Opal</sup> vector.

20.

A transformed host cell comprising the nucleotide sequence of claim 1.

21.

A transformed host cell comprising the synthetic suppressor tRNA molecule of claim 2.

22.

A method for introducing site-specific mutation to a translated protein comprising:

introducing to said cell a nucleic acid sequence which encodes a synthetic suppressor tRNA oligonucleotide, said oligonucleotide comprising:

- A) a human tRNA structural gene sequence comprising no more than twenty 3' flanking residues and no 5' flanking residues, said sequence encoding an anticodon region for pairing with mRNA;
- B) a total length of less than 150 nucleotides;
- C) an anticodon sequence which has been modified to recognize a codon different from that which is originally recognized, wherein said anticodon is one which will pair with said nonsense mutation and said tRNA structural gene sequence encodes an amino acid which is deleted by said nonsense mutation.

23.

The oligonucleotide of claim 22 wherein said anticodon region encodes an nonsense mutation selected from the group consisting of:

amber (TAG), ochre (UAA) and opal (UGA).

24.

The oligonucleotide of claim 22 wherein said tRNA structural gene sequence encodes a serine tRNA.

25.

The oligonucleotide sequence of claim 22 wherein said tRNA structural gene sequence encodes an arginine tRNA.

26.

The oligonucleotide of claim 22 wherein said oligonucleotide has a sequence selected from the group consisting of SEQ ID NOS:1-10, their complements and their functional equivalents.

27.

A method for introducing site-specific mutation to a translated protein comprising:  
introducing to said cell a synthetic suppressor tRNA encoded by the sequence of claim 1.

28.

A method for designing a synthetic suppressor tRNA comprising:

- A) identifying a tRNA sequence of interest;
- B) identifying the anticodon of said tRNA sequence;
- C) designing an alternate anticodon sequence such that a different amino acid is translated in relation to the said anticodon than would normally be;
- (D) synthesizing an oligonucleotide comprising:
  - a) a human tRNA structural gene sequence comprising no more than twenty 3' flanking residues and no 5' flanking residues, said sequence encoding an anticodon region for pairing with mRNA;
  - b) a total length of less than 150 nucleotides;
  - c) an anticodon sequence which has been modified to recognize a codon different from that which is originally recognized, wherein said anticodon is one which will pair with said nonsense mutation and said tRNA structural gene sequence encodes an amino acid which is deleted by said nonsense mutation.

29.

The oligonucleotide of claim 28 wherein said anticodon region encodes an nonsense mutation selected from the group consisting of:

amber (TAG), ochre (UAA) and opal (UGA).

30.

The oligonucleotide of claim 28 wherein said tRNA structural gene sequence encodes a serine tRNA.

31.

The oligonucleotide sequence of claim 28 wherein said tRNA structural gene sequence encodes an arginine tRNA.

32.

A method of treating genetic disease in animals comprising:  
introducing to said animal a suppressor tRNA sequence, said tRNA sequence comprising:

- A) a human tRNA structural gene sequence comprising no more than twenty 3' flanking residues and no 5' flanking residues, said sequence encoding an anticodon region for pairing with mRNA;
- B) a total length of less than 150 nucleotides;
- C) an anticodon sequence which has been modified to recognize a codon different from that which is originally recognized.

33.

The oligonucleotide of claim 32 wherein said anticodon region encodes a nonsense mutation selected from the group consisting of:

amber (TAG), ochre (UAA) and opal (UGA).

34.

The oligonucleotide of claim 32 wherein said tRNA structural gene sequence encodes a serine tRNA.

35.

The oligonucleotide sequence of claim 32 wherein said tRNA structural gene sequence encodes an arginine tRNA.

36.

The oligonucleotide of claim 32 wherein said oligonucleotide has a sequence selected from the group consisting of SEQ ID NOS:1-10, their complements and their functional equivalents.

37.

The method of claim 32 wherein said disease is Xeroderma Pigmentosum.

38.

A method of monitoring transduction of cells comprising:  
introducing to said cells and oligonucleotide vector  
comprising a reporter gene said reporter gene having been  
inactivated by introduction of a nonsense mutation;  
introducing to said cells a suppressor tRNA sequence  
according to claim 1; and  
assaying for reactivation of the reporter gene.

39.

The method of claim 38 wherein said reporter gene is  
selected from the group consisting of: chloramphenicol acetyl  
transferase and green fluorescent protein.

4002427 40001